09/783,896

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L5	L4 and fluorescen$2	12	L5
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L3	L2 and immobiliz$5	13	L3
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L1	immuno same RNA same amplif$7	37	L1

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☑ 1. US 5827661 A. Enhanced detection by nucleic acid amplification, especially of Listeria - uses formation of DNA-RNA hybrids after amplification, and then specific immuno-detection of these. BLAIS, B W. C07H021/04 C12P019/34 C12Q001/68 G01N033/53.

☐ 2. WO 9822624 A1 AU 9854472 A US 5922553 A EP 960213 A1 JP 2001504586 W US 6255060 B1. Detection of specific protein by RNA immuno amplification - useful for detection of tau protein, used for early diagnosis of Alzheimer's disease. EBERWINE, J, et al. C07H021/04 C07K005/00 C12P019/34 C12Q001/68 C12Q001/70 G01N033/53 G01N033/532 G01N033/543.

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Term	Documents
IMMUNO.DWPI,EPAB,JPAB,USPT.	17855
IMMUNOES	0
IMMUNOS	0
IMMUNOE.DWPI,EPAB,JPAB,USPT.	4
RNA.DWPI,EPAB,JPAB,USPT.	54147
RNAS.DWPI,EPAB,JPAB,USPT.	9027
AMPLIF$7	0
AMPLIF.DWPI,EPAB,JPAB,USPT.	49
AMPLIFABLE.DWPI,EPAB,JPAB,USPT.	4
AMPLIFACATION.DWPI,EPAB,JPAB,USPT.	3
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(IMMUNO NEAR5 RNA NEAR5 AMPLIF$7).USPT,JPAB,EPAB,DWPI.	2

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L4: Entry 1 of 2 File: DWPI Oct 27, 1998

DERWENT-ACC-NO: 1998-593985
DERWENT-WEEK: 199850

TITLE: Enhanced detection by nucleic acid amplification, especially of Listeria - uses formation of DNA-RNA hybrids after amplification, and then specific immuno-detection of these

Basic Abstract Text (7):
ADVANTAGE - Many previous detection techniques use each of the three techniques combined above separately (DNA and RNA amplification, immuno-detection), and combining them increases the overall sensitivity and accuracy of detection. PCR alone is unable to amplify minute quantities of DNA, and is sometimes inhibited by media components from which target DNA was extracted. Amplification of RNA alone requires some of analytical step e.g. labelling some of the dNTP's. These labels, e.g. digoxigenin, can inhibit the RNA polymerase. Use of antibodies in the final detection eliminates the need for preparation of nucleic acid hybridisation probes, which can be expensive as they need to be radio-labelled, and are not as sensitive as antibodies. In addition lack of their use eliminates time and expense of having to handle radioactive contaminants.

Standard Title Terms (1):
ENHANCE DETECT NUCLEIC ACID AMPLIFY LISTERIA FORMATION DNA RNA HYBRID AFTER AMPLIFY SPECIFIC IMMUNO DETECT

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L4: Entry 1 of 2 File: DWPI Oct 27, 1998

DERWENT-ACC-NO: 1998-593985
DERWENT-WEEK: 199850

TITLE: Enhanced detection by nucleic acid amplification, especially of Listeria - uses formation of DNA-RNA hybrids after amplification, and then specific immuno-detection of these

INVENTOR: BLAIS, B W

PATENT-ASSIGNEE: KALYX BIOSCIENCES INC (KALYN)

PRIORITY-DATA: 1994CA-2137070 (December 23, 1994)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
US 5827661 A	October 27, 1998		015	C12Q001/68

APPLICATION-DATA:

PUB-NO	APPL-DATE	APPL-NO	DESCRIPTOR
US 5827661A	December 30, 1994	1994US-0366619	Cont of
US 5827661A	September 23, 1996	1996US-0718596	

INT-CL (IPC): C07 H 21/04; C12 P 19/34; C12 Q 1/68; G01 N 33/53

ABSTRACTED-PUB-NO: US 5827661A
BASIC-ABSTRACT:

Nucleic acid detection comprises:

(a) synthesising DNA amplicons complementary to target nucleic acid sequence by a nucleic acid amplification procedure using at least pair of priming oligonucleotides, one of primer of each of the pairs comprising an RNA polymerase promoter sequence;

(b) transcribing and amplifying the amplicons into transcription enhancement products using an RNA polymerase;

(c) capturing transcription enhancement products by forming RNA:DNA hybrids with an immobilised DNA probe; and

(d) detecting the enhancement:capture probe complexes immunochemically using antibodies which are reactive with RNA:DNA hybrids.

USE - The method is use for enhanced detection of DNA sequences, via a nucleic acid amplification procedure (preferably polymerase chain reaction, PCR), especially for detecting pathogens. Minute samples of pathogens (c. 10 cells) cannot be detected effectively by PCR. The minute quantities of product formed by PCR are then transcribed into RNA enhancement products, which further amplifies the target sequences to detectable levels. Detection then takes place with antibodies for DNA:RNA hybrids, which enable detection if the product volume formed is still small, but is specific enough just for this type of product. The method is especially useful for detecting the following pathogens: Listeria monocytogenes, L. innocua, L. ivanovii, L. seeligeri, L. welshimeri, L. murrayi, L. grayi, Streptococcus thermophillus, Lactobacillus casei, Lactococcus lactis, Micrococcus luteus, Enterococcus faecalis, Staphylococcus epidermidis, Bacillus cereus, Bacillus subtilis, Pseudomonas

aeruginosa, Escherichia coli, Salmonella typhimurium, or Yersinia enterocolitica.

ADVANTAGE - Many previous detection techniques use each of the three techniques combined above separately (DNA and RNA amplification, immuno-detection), and combining them increases the overall sensitivity and accuracy of detection. PCR alone is unable to amplify minute quantities of DNA, and is sometimes inhibited by media components from which target DNA was extracted. Amplification of RNA alone requires some of analytical step e.g. labelling some of the dNTP's. These labels, e.g. digoxigenin, can inhibit the RNA polymerase. Use of antibodies in the final detection eliminates the need for preparation of nucleic acid hybridisation probes, which can be expensive as they need to be radio-labelled, and are not as sensitive as antibodies. In addition lack of their use eliminates time and expense of having to handle radioactive contaminants.

ABSTRACTED-PUB-NO: US 5827661A
EQUIVALENT-ABSTRACTS:

CHOSEN-DRAWING: Dwg.0/4

DERWENT-CLASS: A96 B04 D16 S03
CPI-CODES: A12-L04; A12-W11L; B04-E01; B04-E05; B04-E07; B04-F10; B04-G01; B11-C08E5; B12-K04A4; B12-K04F; D05-H04; D05-H09; D05-H10; D05-H11; D05-H12D1; D05-H12D5; D05-H18B;
EPI-CODES: S03-E14H4;

End of Result Set

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L5: Entry 12 of 12 File: USPT Sep 9, 1997

DOCUMENT-IDENTIFIER: US 5665539 A
TITLE: Immuno-polymerase chain reaction system for antigen detection

Abstract Text (1):
A novel system and method for sensitive antigen detection. The system utilizes immuno-polymerase chain reaction in which a specific biotinylated nucleic acid molecule is used as the marker. The biotinylated marker is attached to antigen-antibody complex through a streptavidin-protein A chimeric protein that possesses tight and specific binding affinity both for biotin and immunoglobulin G. A segment of the attached biotinylated marker is amplified by polymerase chain reactions with appropriate primers and the polymerase chain reaction products are detected by agarose gel electrophoresis. The method can detect any antigen and has a greater sensitivity than any existing antigen detection system.

Brief Summary Text (5):
Attempts have been made previously to develop sensitive methods for detection of antigenic, nucleic acid containing entities. For example U.S. Pat. No. 5,077,192 relates to a method for the detection of low levels of medically important organisms such as bacteria, viruses, malignant cells and the like. The abstract of the Japanese application JP 3231151 describes detecting a modified nucleic acid recognized by an antibody added to 5'-terminal site of primer or to DNA amplified by primer. PCT 91/AU91/00131, filed on Apr. 5, 1991, describes a method for capturing target DNA from a sample, amplifying the DNA using a PCR and detecting the amplified DNA.

Brief Summary Text (9):
(a) contacting said antigen with an antibody to form a first complex comprising of antigen and antibody;

Brief Summary Text (11):
(c) conjugating said first complex with a second complex to form a third complex comprising the antigen-antibody-linker-biotinylated marker;

Brief Summary Text (14):
Another aspect of the current invention is a method wherein a linker is protein, peptide, a streptavidin-protein A chimeric protein containing both a biotin-binding domain and an antibody-binding domain, a cross-linked marker nucleic acid-antibody or a biotinylated marker nucleic acid cross-linked to biotinylated antibody by streptavidin or avidin.

Brief Summary Text (15):
Still another aspect of the current invention is a method wherein streptavidin in the chimeric protein binds specifically to biotinylated marker through its biotin-binding domain and protein A binds specifically to the antibody through its immunoglobulin G-binding domain.

Brief Summary Text (17):
(a) contacting said antigen with an antibody to form a first complex comprised of antigen and antibody;

Brief Summary Text (19):
(c) conjugating said first complex with a second complex to form a third complex comprising biotinylated marker-biotin binding domain-antibody-antigen conjugate;

Brief Summary Text (23):

Still another aspect of the current invention is a composition for use in an immunoassay, comprising a peptide or protein having an antibody-binding domain and an amplifiable polynucleic acid bound to said peptide.

Detailed Description Text (2):
The invention concerns an extremely sensitive method and system for detection of antigens by means of specific antigen-antibody-marker-conjugates. The antigen detection system, called immuno-polymerase chain reaction, utilizes a specific DNA molecule as a marker and a bispecific chimeric protein as a linker between the marker molecule and the antigen to be detected while conjugated to an antibody.

Detailed Description Text (3):
There are several novel features involved in this invention. First, a nucleic acid sequence is used as the marker for detection of antigen. Second, antibody-linker-biotinylated marker conjugates are used to attach a marker molecule to an antigen. Third, enzymatic steps such as a polymerase chain reaction are used to amplify signals for detection of specific antigens. Fourth, due to the specificity and efficiency of nucleic acid amplification, the detection sensitivity of the immuno-PCR technology is superior to that of any existing antigen detection system and the method is, in principle, able to detect a single antigen molecule. No method of such sensitivity is currently available. Fifth, a wider variety of antigens can be detected by the immuno-PCR than by other currently available antigen detection systems.

Detailed Description Text (4):
Briefly, in the current invention, a linker molecule with bispecific binding affinity for nucleic acids and antibodies is used to attach a DNA, RNA, DNA/RNA hybrid, or their fragment, analogue or derivative molecule used as a marker, specifically to an antigen-antibody complex, resulting in the formation of a specific antigen-antibody-linker-DNA conjugate. A segment of the attached marker is amplified enzymatically (such as by a polymerase chain reaction with appropriate primers). The presence of specific products of polymerase chain reaction or other amplification methods demonstrates that marker molecules are attached specifically to antigen-antibody complexes and in turn, this indicates the presence of antigen.

Detailed Description Text (5):
A streptavidin-protein A chimera previously synthesized and disclosed in co-pending applications identified above was used as the linker molecule. The chimera has two independent specific binding abilities. One is its binding to biotin, derived from the streptavidin moiety, and the other is its binding to the Fc portion of an immunoglobulin G (IgG) molecule, derived from the protein A moiety. This bifunctional specificity both for biotin and antibody allows the specific conjugation of any biotinylated nucleic acid molecule to antigen-antibody complexes. Other linker molecules, such as any protein, peptide, nucleic acid marker chemically cross-linked to antibodies, or biotinylated marker nucleic acid cross-linked to biotinylated antibodies by streptavidin or avidin may be also advantageously utilized.

Detailed Description Text (6):
In the current invention, a streptavidin-protein A chimera or any other linker that possesses tight and specific binding affinity both for biotin and immunoglobulin G was used to attach a biotinylated marker specifically to antigen-monoclonal antibody complexes that had been immobilized on microtiter plate wells. Next, a segment of the attached marker was amplified by PCR. Analysis of the PCR products by agarose gel electrophoresis after staining with ethidium bromide allowed as few as 580 antigen molecules (9.6.times.10.sup.-22 moles) to be readily and reproducibly detected. Direct comparison with enzyme-linked immunosorbent assays (ELISA) with the use of a chimera-biotinylated alkaline phosphatase conjugate demonstrated that approximately 10.sup.5 times enhancement in antigen detection sensitivity was obtained with the use of immuno-PCR. Given the enormous amplification capability and specificity of PCR, the current immuno-PCR technology has a sensitivity greater than that of any existing antigen detection system and, in principle, is sensitive enough to be applied to the detection of single antigen molecules.

Detailed Description Text (7):
One mode of the immuno-PCR technology of the current invention, in which a specific antibody-DNA conjugate is used to detect antigens, utilizes immobilization of various

amounts of an antigen on the surface of microtiter plate wells. For initial testing, bovine serum albumin (BSA) was used as the antigen because of the availability of both the pure protein and monoclonal antibodies against it. The detection procedure used was similar to conventional enzyme-linked immunosorbent assays (ELISA). Instead of an enzyme-conjugated secondary antibody directed against the primary antibody, as in typical ELISA, a biotinylated linear plasmid DNA (pUC19), conjugated to the streptavidin-protein A chimera, was targeted to the antigen-antibody complexes. A segment of the attached marker was amplified by PCR with appropriate primers and the resulting PCR products were analyzed by agarose gel electrophoresis after staining with the ethidium bromide.

Detailed Description Text (8):
The concept of immuno-PCR of the current invention is shown schematically in FIG. 1. By using linker molecule X which has bispecific binding affinity both for the marker for antibody, a molecule used as a marker can be specifically attached to an antibody-antigen complex. Marker molecules are typically DNA, RNA, DNA-RNA hybrids, their derivatives, fragments, segments or analogues. The attached marker allows the amplification of its segment(s) by PCR with appropriate primers. The enormous amplification capability and specificity of PCR allows the production of large amounts of specific DNA segments as PCR's products. These products can be detected by various methods known and used in molecular biology such as, for example, by agarose gel electrophoresis. The presence of specific PCR products demonstrates that marker molecules are attached to antigen-antibody complexes, indicating the presence of antigen. In addition, the quantitation of PCR products also provides the estimation of the number of antigens (epitope).

Detailed Description Text (9):
A streptavidin-protein A chimera is an ideal molecule to serve as a linker molecule X. Its bifunctional specificity both for biotin and immunoglobulin G (antibody) allows the specific conjugation of a DNA molecule to antigen-antibody complexes upon biotinylation of the DNA.

Detailed Description Text (11):
Linker molecule can be any material which is able to specifically recognize, or which possesses both the antibody-binding domain and also the biotin-binding domain. The linker may be any protein, peptide, nucleic acid marker cross-linked to antibody or biotinylated marker nucleic acid cross-linked to biotinylated antibody by streptavidin or avidin. The most preferred linker is a recombinant streptavidin-protein A chimeric protein.

Detailed Description Text (12):
A recombinant streptavidin-protein A chimeric protein having two biological recognition specificities was used as a linker between an antigen-antibody complex and biotinylated DNA. A gene fusion of streptavidin with protein A encoding two immunoglobulin G (IgG)-binding domains was efficiently expressed in Escherichia coli and the expressed chimeric protein was purified to homogeneity by simple procedures well known in the art. The purified chimeric protein can bind one biotin molecule per subunit and has thus full biotin-binding ability and can also bind one or more immunoglobulin G molecules per subunit. With the specific and tight binding affinity of the streptavidin-protein A chimeric protein both for immunoglobulins and biotin, any biological material containing biotin may be conjugated and/or labeled with immunoglobulin molecules and/or such molecules may be detected. This dual ability of streptavidin-protein A chimera was utilized in the current invention to link the antigen-antibody complex with biotinylated DNA subsequently amplified by polymerase chain reaction.

Detailed Description Text (13):
Two biological recognition specificities of the streptavidin-protein A chimeric protein of the current invention are conferred on that protein by streptavidin which specifically binds biotin with extremely high affinity and by protein A which binds various antibodies, preferably IgG, with high affinity. Since biotin can be easily incorporated into various biological substances, the streptavidin-biotin system offers an avenue by which the second system, namely protein A-bound antibody, such as human or other mammalian IgG, IgG.sub.1, IgG.sub.2, IgG.sub.3, IgG.sub.4, IgM, IgA, SIgA or IgE, can be conjugated with antigens.

Detailed Description Text (17):
A specific marker molecule was used as a marker in the conjugated DNA-linker-antibody-antigen complex. The marker molecule may be DNA, RNA, DNA-RNA hybrid, fragment, segment, their derivative and analogue. The marker DNA, as used in this invention, serves as the entity recognizable and amplifiable by PCR. For the purposes of this invention, the marker DNA is biotinylated. Biotinylated DNA is easily conjugated to the streptavidin moiety of the streptavidin-protein A chimera allowing a specific binding of biotinylated DNA to the chimera.

Detailed Description Text (24):
Conjugation of Antigen with Antibody

Detailed Description Text (25):
Any antigen to be detected is conjugated with its own specific antibody to form an antigen-antibody complex. Antibody can be monoclonal or polyclonal such as various immunoglobulins IgG, IgM, IgA, IgE and their fragments, derivatives and analogues. Antibody can be biologically or chemically produced or genetically engineered.

Detailed Description Text (26):
Any antigen can be detected by the current method as long as it conjugates with antibody which is recognizable by the linker. Antigen may be, among others, a protein, peptide, lipid, carbohydrate, nucleic acid, hapten or their derivatives.

Detailed Description Text (35):
Polymerase chain reaction is the process wherein a specific segment of DNA can be amplified by more than a hundred thousand times relative to nearby nucleotide sequences. In the current invention the enormous amplification capability is used to replicate a segment of the biotinylated DNA attached specifically to streptavidin protein A chimera-antibody-antigen complexes to achieve the highest possible degree of sensitivity in antigen detection. Such high sensitivity will result from million to billion fold amplification of a segment of the biotinylated DNA which is attached specifically to the antigen to be detected.

Detailed Description Text (47):
Detection of a label which has been incorporated directly into marker DNA is performed by methods known in the art. Many labels, which can be incorporated specifically into DNA, are available. They include fluorescent dyes such as for example, ethidium bromide and ethidium homodimer, which are frequently used in molecular biology. Another way is to attach a hapten such as for example, biotin and FITC, to the marker DNA, followed by the detection of the label by using, for example, antibodies against the label.

Detailed Description Text (49):
Detection of Antigen Immobilized on Microtiter Plate by Immuno-PCR

Detailed Description Text (50):
The current method concerns a detection of very small amounts of antigen bound by an antibody, which is conjugated to IgG-binding moiety of protein A in streptavidin-protein A chimera, which is conjugated through its biotin-binding moiety to the biotinylated DNA. A segment of the attached DNA is amplified with PCR and the PCR product is detected by agarose gel electrophoresis.

Detailed Description Text (51):
Typically, the antigen is first conjugated to the antibody to form a first complex and the biotinylated DNA is conjugated to streptavidin-protein A chimera to form a second complex. The first and the second complexes are then conjugated to form the third complex, i.e. biotinylated DNA-streptavidin-protein A chimera-antibody-antigen conjugate.

Detailed Description Text (52):
Because of the availability of pure antigen and of monoclonal antibodies against it, to develop and to test the immuno-PCR method of this invention, bovine serum albumin (BSA) was used as the antigen. The procedures for detecting BSA immobilized on a microtiter plate by immuno-PCR are described below. The detection method and assays

for other antigens are run under the same or slightly modified conditions.

Detailed Description Text (53):
Various amounts (6.4 ng-6.4 ag; 96 fmol-9.6.times.10.sup.-22 mol; 5.8.times.10.sup.10 -5.8 .times.10.sup.2 molecules) of BSA or other antigen in 45 .mu.l of 150 mM NaCl, 20 mM Tris-Cl (pH 9.5), and 0.02% NaN.sub.3 prepared by serial dilution, are placed in wells of a microtiter plate (Falcon 3911, polyvinyl chloride; Becton Dickinson). The microtiter plate is incubated at 2.degree. C.-20.degree. C., preferably at 4.degree. C., for 6-16 hours or overnight, to immobilize BSA molecules on the surface of the wells. The same solution without BSA is used as the control.

Detailed Description Text (54):
The wells are briefly washed several times with Tris-buffered saline (TBS) consisting of 150 mM NaCl, 20 mM Tris-Cl (pH 7.5), and 0.02% NaN.sub.3], and 200 .mu.l of ETBS (TBS plus 0.1 mM EDTA) containing 4.5% non-fat dried milk and 1 mg/ml denatured DNA, such as salmon sperm DNA, are added into each well. The microtiter plate is incubated at 32.degree. -42.degree. C., preferably 37.degree. C. for 40-120 minutes, preferably 80 minutes, to block reactable sites on the surface of the wells to avoid non-specific binding in subsequent steps, and then the wells are washed several times with TETBS (TBS plus 0.1 mM EDTA and 0.1% Tween 20). Into each well is added 50 .mu.l of TETBS containing 0.45% non-fat dried milk, 0.1 mg/ml denatured salmon sperm DNA, and diluted monoclonal or polyclonal antibody. For testing of BSA antigen, 8,000-fold diluted monoclonal antibody against BSA which was mouse ascites fluid; IgG2a; clone BSA-33, obtained from Sigma, is used. The plate is incubated at 18.degree.-26.degree. C., preferably at room temperature (.about.20.degree.C.) for 15-90 minutes, preferably for 45 minutes to allow the antibody to bind to immobilized BSA molecules. The wells are then washed extensively with TETBS to remove unbound antibody molecules, and about 50 .mu.l of TETBS containing 0.45% non-fat dried milk, 0.1 mg/ml denatured salmon sperm DNA, and 140 amol of biotinylated pUC19 conjugated to the streptavidin-protein A chimera are added into each well. The microtiter plate is incubated at room temperature for 20-120 minutes, preferably for 60 minutes, to allow the chimera-pUC19 conjugates to bind to the antigen-antibody complexes. The wells are again washed extensively with TETBS to remove unbound conjugates and again washed briefly with TBS without NaN.sub.3. The microtiter plate is subjected to PCR as described above. After the PCR amplification, each reaction mixture is analyzed by agarose gel electrophoresis. A post-PCR mixture, in amount from 10-30 .mu.l, preferably about 15 .mu.l, is analyzed by standard agarose gel electrophoresis, stained with ethidium bromide.

Detailed Description Text (57):
In other variations of the instant method, marker nucleic acid is attached specifically to targets, not via antigen-antibody binding but by using specific binding ability of a linker. The attached marker nucleic acids are detected by polymerase chain reaction or by other appropriate methods. Examples of such linker-target pairs include receptors and their ligands, enzymes and their cofactors, enzymes and their substrates, and DNA and DNA binding proteins.

Detailed Description Text (58):
FIG. 1 illustrates schematically the concept of the current invention. A streptavidin-protein A chimeric protein, identified as (X), is used as a linker between biotinylated DNA and antibody-antigen complex resulting in the formation of a specific antigen-antibody-linker-DNA conjugate. The streptavidin-protein A chimera possesses specific binding affinity for both DNA and antibody. Biotinylated DNA is used as a marker. Antigen is bound through its epitope, the antigenic determinant on an antigen, to the paratope on an antibody.

Detailed Description Text (59):
A segment of the attached marker DNA of the antigen-antibody-linker-DNA conjugate can be amplified by polymerase chain reaction (PCR) using appropriate primers. Polymerase chain reaction has enormous amplification capability which allows the production of large amounts of specific DNA products (PCR products), which can be detected with great sensitivity by various methods.

Detailed Description Text (60):
The extremely high sensitivity of polymerase chain reaction for a target sequence of

biotinylated DNA, defined by a set of primers, eliminates the generation of false PCR signals derived from other DNA molecules present in samples. The presence of specific polymerase chain reaction products in the sample demonstrates that marker biotinylated DNA molecules are attached specifically to antigan-antibody complexes evidencing the presence of the antigen.

Detailed Description Text (63):
In principle, the immuno-PCR technology can be applied to detection of single molecules. No method is currently available which would have the same degree of sensitivity. In addition, the extremely high sensitivity of the immuno-PCR considerably reduces the amounts of antibodies required resulting in reduced assay costs. This is particularly useful when large amounts of specific antibodies are not available.

Detailed Description Text (67):
Besides the streptavidin-protein A chimera, any molecule, that allows the specific conjugation of a marker DNA to an antibody, can be used. This includes chemical conjugation of a marker DNA to antibody and bispecific antibodies to a primary antibody and a hapten-containing DNA marker.

Detailed Description Text (68):
Using a similar configuration, any antigen molecule, which is efficiently separated from unbound antibody and unbound chimera-nucleic acid conjugates, can be detected by immuno-PCR without the need for modification of the basic procedure. Such antigen molecules include:

Detailed Description Text (69):
i) antigen immobilized on other types of solid supports, such as membranes and beads;

Detailed Description Text (74):
By repeating the cycles of binding antibody to its antigen and binding chimera-DNA conjugates to antigen-antibody complexes, many different DNA markers can be attached to different antigen-antibody complexes. By using specific PCR primers for each DNA marker at the PCR amplification step, many different antigens can be simultaneously detected.

Detailed Description Text (75):
Pre-conjugation of nucleic acid makers to antibodies, i.e., use of antibody-chimera-nucleic acid marker conjugates, should decrease background levels of samples containing molecules that can bind protein A. The current invention is particularly useful for diagnostic purposes. The type and quantity of antigen present in the blood and other bodily fluids is detected by the assay of the current invention. The assay can also be useful for detection of immunodeficiencies allergies.

Detailed Description Text (78):
Detection of Immobilized BSA By Immuno-Polymerase Chain Reaction

Detailed Description Text (79):
This example illustrates a procedure for detection of immobilized BSA by immuno-polymerase chain reaction.

Detailed Description Text (80):
Various amounts (6.4 ng-6.4 ag; 9.6.times.10.sup.-22 mol) of BSA in 45 .mu.l of 150 mM NaCl, 20 mM Tris-Cl (pH 9.5), 0.02% NaN.sub.3, prepared by serial dilutions, were placed in wells of a microtiter plate (Falcon 3911; Becton Dickinson). The microtiter plate was incubated at 4.degree. C. overnight to immobilize BSA molecules on the surface of the wells. The same solution without BSA was used as the control. The wells were briefly washed several times with Tris-buffered saline [TBS;150 mM NaCl, 20 mM Tris-Cl (pH 7.5), 0.02% NaN.sub.3]. Then 200 .mu.l of ETBS (TBS plus 0.1 mM EDTA) containing 4.5% non-fat dried milk and 1 mg/ml denatured salmon sperm DNA was added to each well. The microtiter plate was incubated at 37.degree. C. for 80 minutes to block reactable sites on the surface of the wells to avoid non-specific binding in subsequent steps, and then the wells were washed several times with TETBS (TBS plus 0.1 mM EDTA and 0.1% Tween 20). Into each well, 50 .mu.l of TETBS containing 0.45%

non-fat dried milk, 0.1 mg/ml denatured salmon sperm DNA, and diluted (8,000-fold) monoclonal antibody against BSA (mouse ascites fluid, IgG2a, clone BSA-33; Sigma) was added. The microtiter plate was incubated at room temperature for 45 minutes to allow the antibody to bind to immobilized BSA molecules. The wells were washed extensively with TETBS to remove unbound antibody molecules, and 50 .mu.l of TETBS containing 0.45% non-fat dried milk, 0.1 mg/ml denatured salmon sperm DNA, and 1.4.times.10.sup.-16 mol of biotinylated pUC19 conjugated to the streptavidin-protein A chimera, prepared as below, was added to each well. The microtiter plate was incubated at room temperature for 50 minutes to allow the chimera-pUC19 conjugates to bind to the antigen-antibody complexes, and then the wells were washed extensively with TETBS to removed unbound conjugates. The wells were washed briefly with TBS without NAN.sub.3, and the microtiter plate was subjected to PCR. After the PCR amplification, each reaction mixture was analyzed by 2% agarose gel electrophoresis.

Detailed Description Text (85):
This example illustrates immuno-polymerase chain reaction conditions used for amplification of a segment of a marker DNA of chimera-marker DNA-antibody-antigen complexes.

Detailed Description Text (87):
Following the PCR, post-PCR mixtures were analyzed (15 .mu.l of each) by 2% agarose gel electrophoresis, stained with ethidium bromide.

Detailed Description Text (90):
This example illustrates a method for detection of biotinylated DNA segments conjugated to the streptavidin-protein A chimera further conjugated to antigen-antibody complex. PCR products are analyzed by standard agarose gel electrophoresis.

Detailed Description Text (91):
A PCR amplification reaction mixture obtained in Example 2 (15 .mu.l) was separated on 2.0% agarose gels, and the DNA was stained with ethidium bromide.

Other Reference Publication (2):
Science, vol. 258, 2 Oct. 1992, "Immuno-PCR: Very Sensitive Antigen Detection by Means of Specific Antibody-DNA Conjugates," Takeshi Sano, Cassandra L. Smith, Charles R. Cantor.

Other Reference Publication (7):
Surolia, A., Pain, D., Khan, M.I., Protein A: Nature's Universal Antibody, Trends Biol. Sic., pp. 74-76, (Feb. 1982).

Other Reference Publication (11):
Lowenadler, B.; Nilsson, B.; Abrahmsen, L.; Moks, T.; Ljunggvist, L.; Holmgren, E.; Paleus, S.; Josephson, S.; Philipson, L.; Uhlen, M. Production of Specific Antibodies Against Protein A Fusion Proteins, E.M.B.O. J 5:2393-2398 (1986).

Other Reference Publication (12):
Sano, T.; Cantor, C.R., A Streptavidin-Protein A Chimera that Allows One-step Production of a Variety of Antibody Conjugates, Bio/Technology, 9:1378-1381 (1991).

CLAIMS:

7. A method according to claim 1 wherein said receptor is an antibody and said ligand is an antigert or hapten.

10. A method according to claim 1 wherein said receptor is an antigen or hapten and said ligand is an antibody.

13. A method for detecting a ligand in a sample, said method comprising steps:

(1) contacting a sample comprising a ligand with a conjugate comprising a non-nucleic acid receptor capable of specifically binding said ligand and a nucleic acid marker comprising a predetermined nucleotide sequence to form a specifically bound complex of said ligand and said conjugate, wherein said receptor is an antibody and said ligand

is an antigen or hapten;

(2) specifically detecting the presence or absence of said nucleic acid marker of said complex, wherein said specifically detecting step comprises amplifying said nucleic acid marker in a polymerase chain reaction,

wherein the presence of said nucleic acid marker indicates the presence of said ligand in said sample.

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